Brit. J. Pharmacol. (1946), **1**, 278.

THE ANTIHISTAMINE SUBSTANCE 2786 R.P.

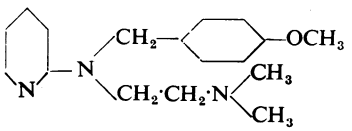
BY

P. B. DEWS AND J. D. P. GRAHAM

From the Department of Pharmacology, Oxford

(Received Oct. 11, 1946)

Bovet and Walthert (1944) have described the properties of a substance which is known as 2786 R.P., and called by them neoantergan ; it is *N*-dimethylaminoethyl *N*-*p*-methoxybenzyl-α-aminopyridine.



This substance powerfully inhibits the action of histamine in animals and has been used clinically with success in the treatment of chronic urticaria and other conditions like hay fever. Benadryl (dimethylaminoethylbenzhydryl ether hydrochloride) was introduced by Loew, Kaiser, and Moore (1945) a year later, and Dr. H. O. Schild informs us that when tested on the guinea-pig ileum in comparison with benadryl, neoantergan is 7 to 18 times more powerful against histamine, depending on the length of contact with the ileum, and 60 to 70 times less powerful against acetylcholine.

We have examined neoantergan by several of the methods used by Bovet and Walthert, and have confirmed their main results. We have also examined neoantergan by several other methods which will now be described.

Experimental Observations

Action on isolated auricles.—Very little stress was given by Dale and Laidlaw (1910), in their original description of the properties of histamine, to the stimulant action of histamine on cardiac tissue. The stimulant action was first demonstrated by Gunn (1926). It is not, however, nearly so striking in the isolated heart perfused by Langendorff's method as when the auricles of the rabbit heart are dissected and suspended in a bath of well-oxygenated Ringer's solution at 28° C. Oxygenation is provided by a gas distribution tube at the bottom of the vessel. In these circumstances the addition of histamine in such amount that

the concentration of base is 1 in 1 million causes a large augmentation of the beat; the effect is seen twice in succession in Fig. 1. Neoantergan was then

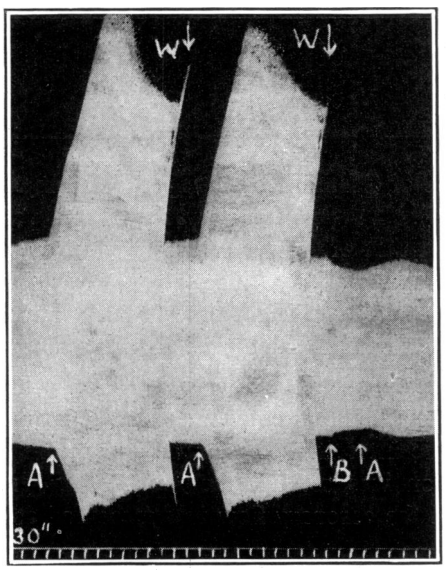


FIG. 1.—Isolated auricles of rabbit heart. At A, histamine was added to the bath (0.1 mg. to 100 ml.). Note the great increase of amplitude. At B, 0.5 mg. neoantergan was added, after which the addition of histamine was without effect.

added to the bath, and in its presence the addition of histamine was without effect.

Action on the driven auricles.—Dawes (1946) has recently described a preparation to measure the action of quinidine on cardiac tissue. The isolated auricles of the rabbit are arranged in contact with electrodes so that they can be made to beat at an imposed rate. A maximum rate can be determined beyond which they cannot follow the stimuli applied, and this maximum rate is reduced when the auricles are exposed for a given length of time to a given concentration of quinidine, or of a substance having a similar action. We have tested neoantergan by this method, and have found that it is approximately twice as active as quinidine. Table I gives the results obtained in one of the experiments. A comparison was undertaken of the toxicity of neoantergan for mice with that of quinidine, both substances being given by intraperitoneal injection. It was found that neoantergan was approximately twice as toxic as quinidine, the LD50 of neoantergan being approximately 120 mg. per kg., whereas that of quinidine was approximately 225 mg. per kg. (About 30 mice were used for each substance.) Neoantergan is thus of equal value to quinidine for its action on the heart; it is twice as active, but twice as toxic. It should be noted that

TABLE I

COMPARISON OF NEOANTERGAN WITH QUINIDINE ON THE ISOLATED AURICLES OF THE RABBIT

Substance	Dose in mg. in 100 ml. bath	Percentage reduction in maximal rate
Quinidine	0·25	7·9
	0·5	17·2
Neoantergan ..	0·125	8·3
	0·25	15·3

the figure for the LD50 of quinidine by intraperitoneal injection differs from that given by Dawes (1946). Dawes's figure was 135 mg. per kg. The difference between our figure and that of Dawes serves to illustrate the point that figures for toxicity have no general validity, since they can vary by as much as 100 per cent in the same laboratory at different times. Such figures are only of use for comparing two substances at the same time (compare Burn and Greville, 1931).

Action on coronary flow.—Histamine is well known to produce a dilatation of the coronary vessels of the cat, and since histamine is here relaxing smooth muscle we were interested to examine the action of neoantergan. The Langendorff preparation was set up, the heart being perfused with Ringer's solution at 37° C. and the coronary flow was measured as the outflow from the

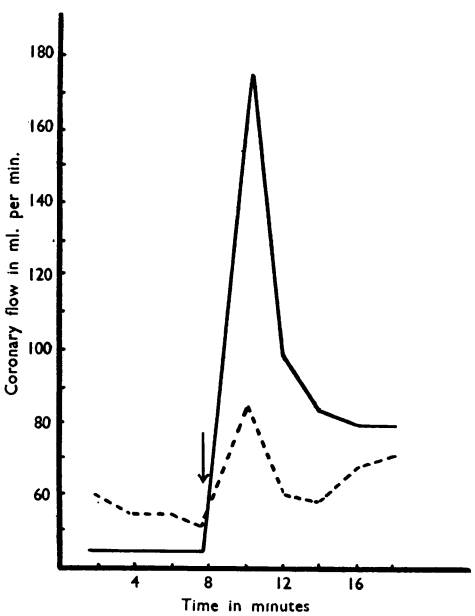


FIG. 2.—Abscissae: time in min. Ordinates: coronary flow in ml. per min. recorded in the heart–lung preparation of the dog. At the arrow 0.1 mg. histamine was injected into the sup. vena cava with the effect shown in the continuous line. Later 0.1 mg. neoantergan was injected followed 1 min. later by 0.1 mg. histamine. The effect of the histamine was that shown in the broken line.

heart. The results in different experiments were similar, and the following is an example. The injection of 25 μg. histamine caused an increase in coronary flow from 4.4 ml. per min. to 12.0 ml. per min. When this effect had finally passed off in about 6 min., neoantergan was injected in a dose of 25 μg. and followed by 25 μg. histamine. The coronary flow increased to 6 ml. per min. only, and returned in 2 min. to the previous rate.

A similar experiment was carried out in the heart–lung preparation of the dog, in which the coronary flow was recorded by inserting a Morawitz cannula in the coronary sinus. The result is shown in Fig. 2. These experiments show that neoantergan reduces the effect of histamine in relaxing the smooth muscle of the coronary arteries.

Action on blood vessels.—The vessels of the rabbit's ear perfused with Ringer's solution by the method of Gaddum and Kwiatkowski (1938) are constricted by small doses of histamine. We have found that this constrictor action is extremely sensitive to the presence of neoantergan and disappears when very small amounts are injected. Fig. 3 shows that the constrictor action of 0.4 μg.

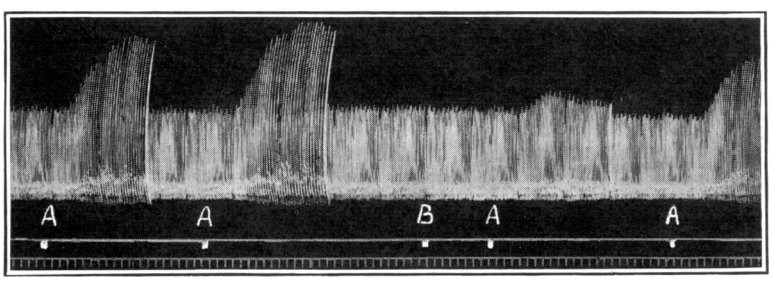


FIG. 3.—Perfusion of rabbit's ear with Ringer's solution; outflow recorded by Gaddum's drop-timer. At A, 0.4 μg. histamine injected. At B, 0.01 μg. neoantergan injected.

histamine was almost abolished by the previous injection of 0.01 μg. neoantergan, and we have observed that a significant reduction was produced by 0.001 μg. neoantergan.

In experiments in which blood flowed through the vessels, the effect of neoantergan on histamine was much less. In the hind-leg of the dog perfused by a pump, the vasodilator action of 3–5 μg. histamine was in two experiments greatly diminished and in a third little affected by the previous injection of 10 μg. neoantergan. In the cat anaesthetized with chloralose, when the spleen volume and the blood pressure were recorded, the intravenous injection of neoantergan abolished the action of histamine on the spleen, but not the depressor action on the blood pressure, even when a dose as large as 10 mg. was injected. (See Fig. 4.) On the other hand, in cats anaesthetized with ether, the injection of 2.5 mg. neoantergan greatly diminished the action of histamine on the blood pressure, so that even 80 μg. produced less fall than 10 μg. previously.

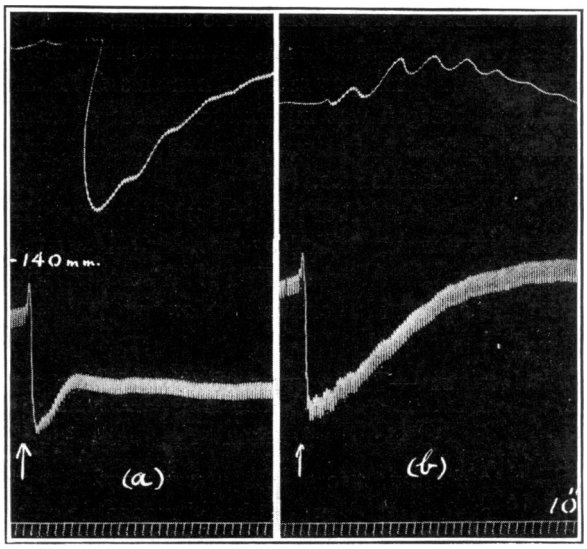


FIG. 4.—Cat ; chloralose. Upper record is spleen volume ; lower record is blood pressure. At the arrow 10 μg. histamine was injected intravenously. Between (a) and (b) 2.5 mg. neoantergan was injected. Note the abolition of the action on the spleen, while the blood pressure effect remains.

In the rabbit anaesthetized with urethane, the injection of 5 mg. neoantergan somewhat reduced the duration of the fall of blood pressure produced by 0.25 mg. histamine, but wholly abolished the stimulant action of this dose of histamine on the respiration.

Action on the uterus.—The common description of histamine as a stimulant of smooth muscle not only neglects the inhibitory action of histamine on the smooth muscle of the coronary vessels of the cat and dog, but also the inhibitory action on the uterine muscle of the rat. We have already seen that the inhibitory action on the coronary vessels is reduced by neoantergan ; we have failed, however, to observe that neoantergan, in concentrations which have no effect on the uterus, reduces the inhibition of the rat uterus by histamine. This result confirms Bovet and Walthert (1944), but we do not confirm their statement that neoantergan has little antihistamine action on the guinea-pig uterus. Fig. 5 illustrates this effect produced by neoantergan in a concentration of 1 in 500 million.

Action as a spasmolytic.—Neoantergan is an antagonist of acetylcholine on the isolated rabbit intestine, though its effect is slight. The stimulant action of 5 μg. acetylcholine was reduced to less than half by the presence of 0.5 mg. neoantergan in a bath of 100 ml. This atropine-like effect is feeble compared with its antihistamine action on guinea-pig ileum, in which tissue the stimulant action of 1 μg. histamine is reduced to less than half by 0.1 μg. neoantergan.

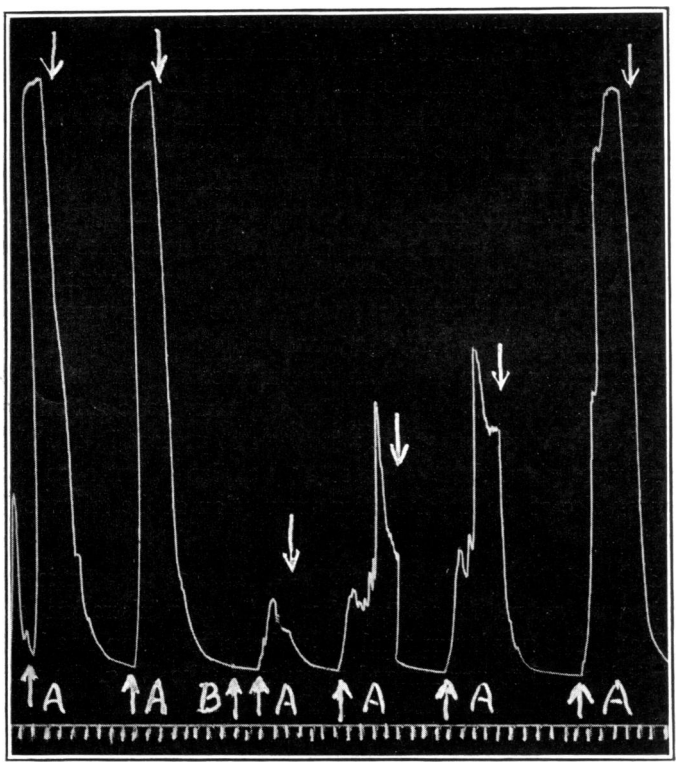


FIG. 5.—Isolated uterus of guinea-pig in bath of 100 ml. At A, 3 μg. histamine added to the bath. At B, 0.2 μg. neoantergan added. The bath was changed as soon as each contraction passed its maximum.

Local anaesthetic action.—Since neoantergan has some atropine-like action, though very little, it was also tested for local anaesthetic action, using the guinea-pig intracutaneous test as described by Bülbring and Wajda (1945). After preliminary observations had shown that a local anaesthetic action was present, a comparison was made with procaine, in which solutions of neoantergan of strengths 0.06, 0.12, and 0.5 g./100 ml. were compared with solutions of procaine of strengths 0.25, 0.5, and 1.0 g./100 ml. The mean result was that neoantergan is 3.1 times as active as procaine by this test.

Analgesic action.—Neoantergan was tested for analgesic action by the method described by Thorp (1946). One group of rats was injected with pethidine, one group was injected with neoantergan, and one group was injected with saline. After determining the threshold for stimulation of the tail, the injections were given intravenously into the tail vein. Rats injected with 5.0 mg. per kg. pethidine then required a much stronger stimulus, the mean increase of threshold being 90.2 per cent.

The dose of neoantergan was as much as 40 per cent of the lethal dose, assuming that the LD50 given by Bovet and Walthert for mice is applicable to rats. This figure is 30 mg. per kg., and the rats were given 12 mg. per kg. The injection caused a slight but not significant increase in the threshold. Each group contained 5 rats, and the same general result was obtained in four separate experiments. Since in each of the last two experiments there was a rather greater rise of threshold in the rats injected with neoantergan than in those injected with saline, it is possible that neoantergan possesses some analgesic action, though, if so, it is feeble. There was, however, great variation among the rats in a group, and many observations would have been necessary to establish that the small rise in threshold was significant.

Further observations were made in which neoantergan was injected subcutaneously. Again there was evidence of a slight analgesic action in some rats when a dose of 30 mg./kg. was given; it was not present in all. When doses of 100 mg./kg. were given there was not only analgesia but a general narcotic effect. The mean rise of the threshold for stimulation of the tail was 80 per cent after one hour.

Effect of daily administration on growth.—Since neoantergan is intended for clinical use it was tested to see whether daily administration exerted any deleterious effect. The maximum total daily dose for a man has been taken as 1.0 g., though it is usual to recommend patients to take 0.1 g. three times a day

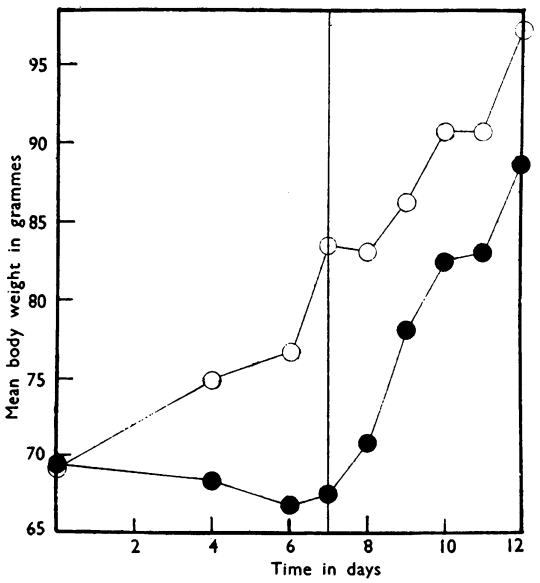


FIG. 6.—Abscissae: time in days; ordinates, mean body weight in grammes. White circles show the increase in body weight of 5 control rats; black circles show the body weight of 5 rats given 25 mg./kg. neoantergan twice daily by subcutaneous injection, for 7 days. After the seventh day the injections were stopped.

at first. This dose is then doubled, and may be recommended four times a day; or again, the dose may be trebled. A dose of 1.0 g. for a man of 70 kg. is equivalent to 14 mg./kg.

The effect of giving a total daily dose of 50 mg./kg. by subcutaneous injection was determined in a group of 5 young rats of 60–70 g. The dose was divided into two parts, half being given in the morning, and half in the evening. Fig. 6 shows the arrest in growth when this dose was given for 7 days, and also shows that when the injections were stopped the rats at once began to grow at the same rate as the group of 5 control rats. The cause of the arrest of growth may have been a decline in the amount of food eaten, though we do not know this; it was probably not due to a toxic effect on the liver or kidney, since growth restarted at once when injections stopped. A second experiment was then carried out in which the total daily dose was 16 mg. per kg. In this experiment there was no effect on the growth of the 5 injected rats as compared with the 5 control rats. Thus a dose equivalent to the maximum human therapeutic dose, when given in two injections daily for 11 days, did not affect the growth of young rats.

Discussion

Perhaps the most striking method of demonstrating the effect of antihistamine substances is to use them to protect guinea-pigs against the action of histamine sprayed into the air from an atomizer. We have used a box with a glass top similar to that described by Bovet and Walthert (1944), in which the guinea-pig is placed. The box is about 12 in. square and 6 in. deep. The nozzle of an atomizer is placed in a small hole in the floor of the box, and a solution of 1 or 2 per cent histamine (base) is sprayed into the box. If a series of guinea-pigs is placed in the box, one at a time, it is seen that they become asphyxiated and collapse in about 90 sec. If they are promptly removed from the box, they recover. The injection under the skin beforehand of 1 mg. neoantergan gives complete and long-lasting protection against the histamine, and we have confirmed Bovet and Walthert's statement that as little as 0.1 mg./kg. gives considerable protection.

The antihistamine action is also easy to demonstrate if a 1 in 1,000 solution of neoantergan is mixed with 1 in 1,000 histamine, and a drop is put on the forearm. If a prick is made through the drop, very little effect of histamine is seen.

Dawes (1946) has pointed out that substances having a quinidine-like action possess other properties in addition. Local anaesthetics, such as procaine and amethocaine, have a quinidine-like action, and so have spasmolytics such as "syntropan" and "trasentin." Substances with an analgesic action like papaverine and pethidine also have a quinidine-like action. We are now able to extend the list to include antihistamine substances, for neoantergan has a quinidine-like action too. It is becoming clearer that there is a large group of substances which possess in common all these different properties, though in

very varying degrees. Neoantergan is not only an antihistamine substance which possesses a quinidine-like action, but it is also a local anaesthetic, and it has a feeble action as an antagonist to acetylcholine on the intestine of the rabbit. It appears to have a slight action as an analgesic which is detectable by the test on the tail of the rat. The chemical relation of neoantergan to other substances with similar properties is not immediately obvious, but many other substances contain a chain resembling dimethylaminoethyl, and also a group corresponding to *p*-methoxybenzyl. In " benadryl " the linkage of the two is effected as an ether. In neoantergan the linkage is through α-amino pyridine ; this is certainly likely to be less toxic than a linkage through aniline, as in the earlier compound antergan (Halpern, 1942).

SUMMARY

1. The properties of the antihistamine substance 2786 R.P., called by Bovet and Walthert neoantergan, have been examined, and the statements of these authors have, in the main, been confirmed. We observed, however, more antihistamine action on the guinea-pig uterus and less on the blood pressure of an animal anaesthetized with chloralose.

2. In addition the following effects have been observed. Neoantergan is a local anaesthetic three times as potent as procaine. It has a quinidine-like action on the auricle, twice as strong as that of quinidine. It abolishes the stimulant action of histamine on cardiac tissue, the dilator action on coronary vessels, and the constrictor action on the vessels of the rabbit ear. It has some analgesic action. When the maximum daily therapeutic dose calculated per kg. is given to young rats daily for 11 days, their growth rate is unaffected, though three times as much causes an arrest of growth.

Our thanks are due to Prof. J. H. Burn for directing this work. The work was done during the tenure by one of us (J. D. P. Graham) of an I.C.I. fellowship awarded by the University of Glasgow. We are indebted to Dr. D. Bovet of the Institut Pasteur for a supply of the material. We also received some from Messrs. May & Baker, Ltd.

REFERENCES

Bovet, D., and Walthert, F. (1944). *Annal. pharmaceut. franc.*, Suppl. No. 4.
Bülbring, E., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 78.
Burn, J. H., and Greville, G. D. (1931). *J. Pharmacol.*, **43**, 645.
Dale, H. H., and Laidlaw, P. P. (1910). *J. Physiol.*, **41**, 229.
Dawes, G. S. (1946). *Brit. J. Pharmacol.*, **1**, 90.
Gaddum, J. H., and Kwiatkowski, H. (1938). *J. Physiol.*, **94**, 87.
Gunn, J. A. (1926). *J. Pharmacol.*, **29**, 325.
Halpern, B. N. (1942). *Arch. internat. pharmacodyn.*, **68**, 339.
Loew, E. R., Kaiser, M. E., and Moore, V. (1945). *J. Pharmacol.*, **83**, 120.
Thorp, R. H. (1946). *Brit. J. Pharmacol.*, **1**, 113.